ber at room temperature offers remarkable advantages, as the radioactivity measurements remain unaffected by operating the chromatograph at different temperatures.

The sensitivity of such a continuous radioactivity measurement device is proportional to the ratio between the chamber volume and the gas flow rate. However, the volume of the ionization chamber is conditioned by the need (among others) to obtain a reasonable resolution of the elution peaks, comparable to that of the thermoconductivity cell in the chromatograph (in our case a Fractovap model B, Società C. Erba). A satisfactory compromise was attained by using a 100-ml ionization chamber calibrated at (total) carrier gas flow rate of 10 lit./hr. To avoid the eddying and mixing of the gases in the ionization chamber, the inlet tube is connected to the top center of the cylindrical chamber body by means of a joint of gradually increasing diameter, thus reducing the speed and eliminating the turbulence of the gas current. The stainless steel chamber may be easily dismantled for decontamination without affecting its calibration. It is mounted on a vibrating reed electrometer (model 31, Applied Physics Corp.) connected to a potentiometric recorder synchronized with that of the Fractovap. When one applies the measuring technique involving the current leakage through a calibrated high resistance, with a 10^{11}-ohm resistor and an input capacity of about 10^{-11} farad, the responses of the electrometer are adequately fast, 63 percent being within 1 second and 85 percent within 2 seconds (*6*). On leaving the ionization chamber, the gas is made to pass through a system of traps cooled with liquid nitrogen, in order that radioactive fractions may be recovered separately. The apparatus is diagramed in Fig. 1.

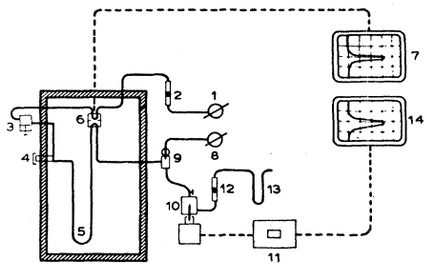

Fig. 1. Apparatus used in the study. (1) Gas inlet regulating valve; (2) carrier gas flowmeter; (3) gaseous samples introduction device; (4) liquid samples introduction device; (5) column; (6) thermoconductivity cell; (7) thermoconductivity cell recorder; (8) dilution gas regulating valve; (9) mixer; (10) ionization chamber; (11) electrometer; (12) chamber flowmeter; (13) liquid nitrogen trap system; (14) electrometer recorder.

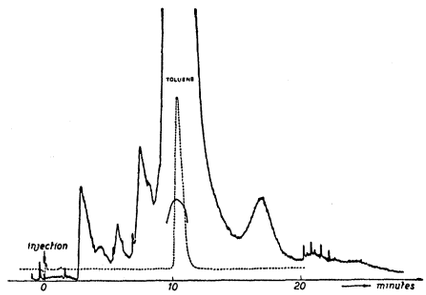

Fig. 2. Analysis of tritiated toluene. Column: polyethylene glycole 400 on celite, length 1 m, internal diameter 4 mm; temperature: 105°C; carrier gas nitrogen; flow rate 1.5 lit./hr in the column, 10 lit./hr in the ionization chamber.

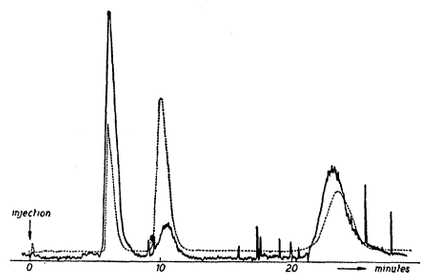

Fig. 3. Analysis of a mixture of benzene, toluene, and chlorobenzene. Conditions were the same as in Fig. 2.

A typical analysis of tritiated toluene, labeled by the Wilzbach method (*7*), is shown in Fig. 2. The dotted tracing refers to the thermoconductivity output; the solid tracing, to the electrometer response. An interesting feature of this analysis is the absence of impurities detectable by means of gas thermoconductivity, while an appreciable percentage of the total radioactivity is given by carrier-free compounds other than toluene, probably by partially hydrogenated benzene and toluene (*8*), as shown in the electrometer tracing. In Fig. 3 is reported the analysis of a mixture of tritiated benzene, toluene, and chlorobenzene, previously purified by means of vapor phase chromatography and hence free of radioactive impurities. The sensitivity of the apparatus employed allows the detection of tritiated compounds having a minimum activity of some mμc, and the measurement of activities of about 20 mμc. Quantitative analyses have been carried out with the use of weighed quantities of compounds having known specific activity. The standard samples have been analyzed under very different operating conditions in the chromatograph: temperature ranging from 70° to 135°C, flow rate from 0.5 to 3.5 lit./hr, and sample size from 0.1 to 10 mg. The calibration has been found to be constant within 2.4 percent.

The method of analysis described here can be extended, without any modification, to volatile organic compounds labeled with other weak β-emitters, as C^{14} and S^{35} (*9*).

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19 October 1959

Morphologic Observations on Trachoma Virus in Cell Cultures

Abstract. When cultures of cells derived from the entoderm of the chick embryo were infected with trachoma virus, cytoplasmic inclusion bodies composed of viral particles were easily demonstrated. The inclusions are similar to those found in stained smears from trachomatous eyes and they developed in a sequence characteristic for this group of viruses. This method of culture appears to offer a valuable additional tool for study of the trachoma agent.

Several recent isolations and successful serial passages of morphologically typical trachoma virus in embryonated eggs (*1*) have for the first time provided a constant source of this virus for detailed examination in the laboratory. This report describes the use of a cell-culture system in which growth of a trachoma virus isolate readily occurs, as indicated by formation of typical inclusion bodies. To our knowledge, no previous report of successful culture of the trachoma virus in vitro has appeared.

The virus used represented the 10th and 11th yolk sac passages of strain No. TW10, obtained from a case of trachoma and isolated in the laboratory of J. Thomas Grayston at the Naval Medical Research Unit No. 2, Taipei,

Taiwan. It was supplied to us by Grayston as a lyophilized preparation of the 5th yolk sac passage.

For inoculation of tissue cultures the virus was partially purified by a procedure adapted from methods found useful for rickettsias (*2*) and for psittacosis virus. It consists of trypsinization of a heavy emulsion of infected yolk sacs, concentration of virus and separation from lipids by high-speed centrifugation, and further removal of extraneous materials by low-speed centrifugation after resuspension in medium containing bovine albumin (0.6 percent by volume) and celite (10 percent of the weight of the original yolk sacs).

The tissue cultures were prepared by a method originally used for culture of feline pneumonitis and mouse pneumonitis viruses (*3*) and since found suitable for culture of other viruses of the psittacosis group and for rickettsias (*4*). The method consists of explanting the entodermal layer of 4-day chick embryos to cover slips and incubating at 36°C under a medium composed of Hanks' balanced salt solution, 75 percent, and chicken serum, 25 percent, without antibiotics. A monolayer of large epithelial cells develops within a few days. Previous investigations in this laboratory (*5*) have shown that psittacosis virus, as well as *Rickettsia prowazeki*, can be detected in much higher dilutions in these cultures if the viral particles are centrifuged onto the cell layer.

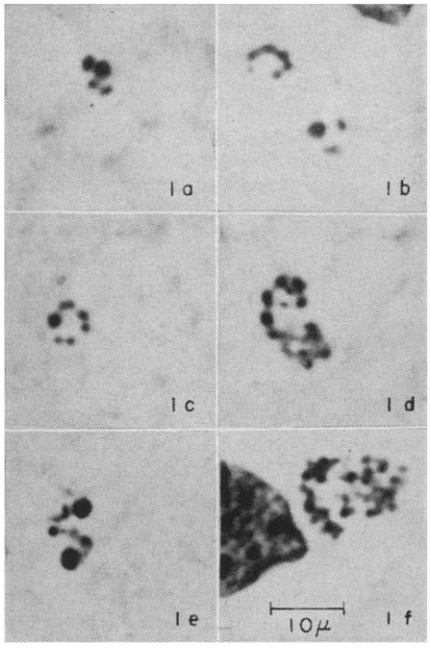

Fig. 1. Virus of trachoma cultivated in entodermal cells of chick embryo. (*a–d*) Initial bodies 18 hours after inoculation, showing compact groups and ring arrangement. (*e*) Plaques and smaller forms at 24 hours. (*f*) Cluster of viral particles at 30 hours. (× 1950)

For this purpose the explants were placed on circular cover slips 12 mm in diameter and incubated in flat-bottomed culture tubes under 0.5 ml of the medium described above. On the 6th or 7th day the fluid was replaced by fresh medium containing 5 percent of virus preparation of the desired dilution. The infected tubes, as well as uninoculated control tubes, were then centrifuged in the horizontal position at 3000 rev/min for 1 hour in a refrigerated centrifuge at 20°C. The fluid was again replaced with fresh medium, and the tubes were returned to the incubator. At selected intervals the medium was removed from groups of tubes and the explants were washed with balanced salt solution, fixed with methyl alcohol, and stained by the May-Grünwald-Giemsa method.

Intracytoplasmic viral inclusion bodies were recognized in preparations fixed as early as 18 hours after inoculation and were seen in varying stages of development through a 72-hour interval. The structures observed were similar to those found in direct smears from patients and were characteristic of this group of viruses (Figs. 1 and 2). The figure legends provide brief descriptions of the morphologic types seen. It is clear that this virus goes through a developmental cycle similar to that of related viruses such as psittacosis and lymphogranuloma venereum.

The maturing inclusions possess a considerable degree of rigidity, as indicated by the rarity of instances of departure from the usual circular or oval outline. The inclusions are often seen to distort the nucleus (Figs. 1*f* and 2*a*) and in turn are only slightly modified by it. In this regard trachoma virus resembles mouse pneumonitis virus cultured in entodermal cells (*3*). By contrast, the virus is very different in appearance from the viruses of feline pneumonitis (*3*) and psittacosis (*6*). In the latter cases the inclusion bodies are quite irregular in shape, occupying space between and around cytoplasmic vacuoles.

Although many cells infected with trachoma virus are seen in satisfactory preparations, the over-all incidence of such cells is low, and no appreciable destruction of the cell culture occurs. In fact, individual cells appear to be little affected by the presence of an inclusion body, at least before the final stages of viral development. There was little or no evidence for a second cycle of viral growth in these cultures, even when they were recentrifuged at times when infective elementary bodies were expected to be present in the fluid. However, the infectivity of the cultures was demonstrated by passage of either culture fluid or trypsinized cells to the yolk sac of embryonated eggs.

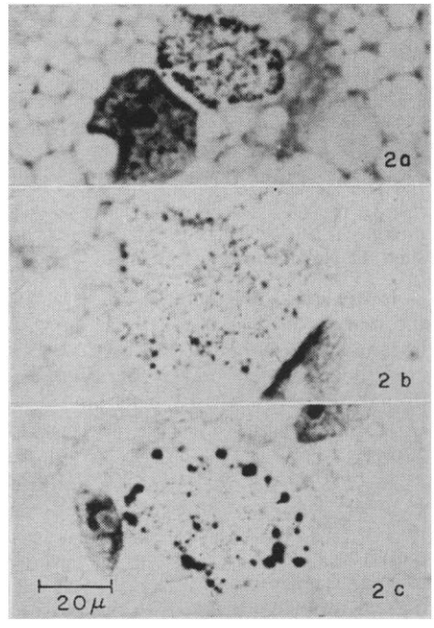

Fig. 2. Virus of trachoma cultivated in entodermal cells of chick embryo. (*a*) Vesicle, at 48 hours. (*b*) Vesicle, at 60 hours, containing numerous elementary bodies. (*c*) Vesicle, at 60 hours, containing elementary bodies and plaques. (× 980)

Although our first studies by this method of culture dealt principally with morphology, we can predict, by analogy with other similar viruses, that this technique will be of value for other types of study as well. Among these are quantitation by means of an infected cell count, as performed with feline pneumonitis virus (*3*), and observations on drug susceptibility as reported with psittacosis virus (*6*).

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